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REVIEW ARTICLE

The Late Na^+ Current - Origin and Pathophysiological Relevance

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In excitable tissues, voltage-dependent Na^+ current (I_{Na}) is best known for supporting autoregenerative depolarization and impulse propagation. Its transient component (I_{NaT}), which is large and terminated within several milliseconds by channel inactivation, fulfils this role. Nevertheless, I_{Na} also includes a smaller sustained component, i.e. one persisting during prolonged membrane depolarization, which contributes to repolarization course. Sustained I_{Na} implies slow or incomplete inactivation of a proportion of the Na^+ channels activated during the action potential upstroke. Several mechanisms may underlie this phenomenon and contribute to arrhythmogenesis in different conditions.

Sustained Na^+ Currents

Window Currents

Truly steady-state currents may flow through otherwise inactivating channels if membrane potential is maintained in a restricted range ("window"). These currents are commonly referred to as "window currents". The window corresponds to the region of overlap between "activation" and "inactivation" curves, in which the probabilities of channels to be open and not inactivated are both greater than 0 at steady-state. A window is normally present in cardiac myocytes for I_{CaL} and, to a lesser extent for I_{Na} (Fig. 1), but it may be enhanced, or shifted to different potentials, by channel mutations (e.g. the Nav1.5 ΔKPQ mutation) [1].

When, during repolarization, membrane potential moves into the "window", a fraction of channels may recover from inactivation and immediately reactivate. The resulting depolarization supports autoregenerative activation which, albeit concerning a small fraction of total channels, may substantially distort membrane potential course (early afterdepolarizations, EADs) and even trigger propagated activity [2, 3]. The I_{Na} window is narrow and more negative than the plateau phase in normal ventricular myocytes; thus, in the latter I_{CaL} window more likely accounts for EADs generation [4] (Fig. 1). I_{Na} window, somewhat more prominent in normal Purkinje myocytes [5, 6], may be widened in all cell types by Na^+ channel mutations.

A further mechanism of I_{Na} enhancement during repolarization is provided by an increase in the rate of channel recovery from inactivation relative to deactivation one. This mechanism, observed in some NaV1.5 mutations, is referred to as "reactivation under non-equilibrium conditions" [7]. This is because the excess Na^+ current can be observed only during dynamic changes of membrane potential (non-equilibrium). This is not a proper steady-state component and, notably, it can escape detection by the standard "voltage step" clamp protocols used to assess the functional phenotype of channel mutations.

Late Na^+ Current (I_{NaL})

Figure 2a shows that sustained I_{Na}, identified as current sensitive to blockade by tetrodotoxin (TTX), exists also at

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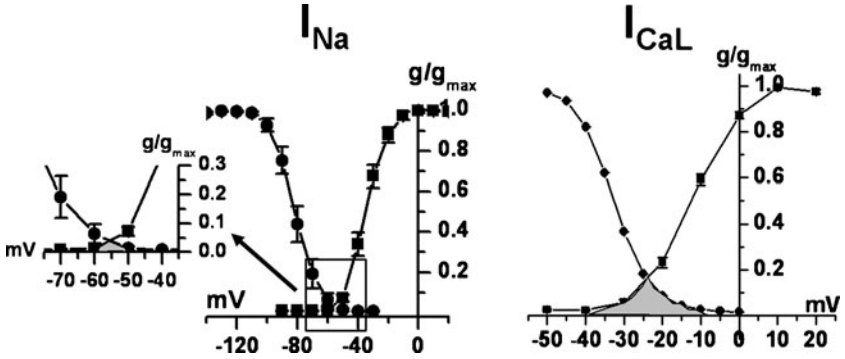

Fig. 1 Comparison of the membrane potential "window" for I_{Na} and I_{CaL} in rat ventricular myocytes. Steady-state activation and inactivation curves for I_{Na} (*left*) and I_{CaL} (*right*). The "window", i.e. the membrane potential range in which activation and inactivation overlap, is filled in grey. I_{Na} window (zoomed in the inset) is smaller and at more negative potentials than I_{CaL} one. (from Rocchetti et al. unpublished)

potentials positive to the I_{Na} window (−65 to −50 mV; shown in in Fig. 1a for the same cell type). This current is provided by a component named "late" Na^+ current (I_{NaL}). I_{NaL}, much smaller than I_{NaT} in normal ventricular myocytes, may be enhanced by more than 3-fold under pathological conditions [8]. Single-channel analysis reveals that the channel gating underlying I_{NaL} is complex and, in addition to late "scattered" openings, it includes a "burst mode" not observed during the transient component of I_{Na} (I_{NaT}) [9, 10]. This led to hypothesize that I_{NaL} might be carried by channel variants other than the one prevailing in cardiac myocytes (NaV1.5), whose expression would be enhanced under pathological conditions (a contribution of NaV1.8 channels to I_{NaL} in mouse and rabbit cardiomyocytes has been indeed reported [11]). However, I_{NaL} with all its gating modes, can be detected by expression of Nav1.5 channels in cell lines (Fig. 2b), where no other Na^+ channel isoforms are present [10, 12]. This proves that I_{NaL} can be carried by the same molecular entity accounting for I_{NaT}; according to this view, I_{NaL} enhancement reflects a gating abnormality. Observations crucial for the development of this interpretation came from single channel analysis of a mutation (ΔKPQ) associated with LQT3 syndrome [13]. The mutant channel had increased probability of burst openings during sustained depolarization, a behaviour interpreted as instability of the inactivated state [1, 13]. The interpretation of I_{NaL} enhancement as a gating abnormality of Nav1.5 channels may hold true also for acquired conditions, as proven in the case of heart failure [14–16]. Nevertheless, we cannot rule out that changes in isoform expression may contribute in some of the diverse conditions in which I_{NaL} enhancement occurs.

Because of recovery from inactivation upon repolarization takes some time, I_{NaT} availability depends on the interval between excitations. This is true also for I_{NaL}, thus

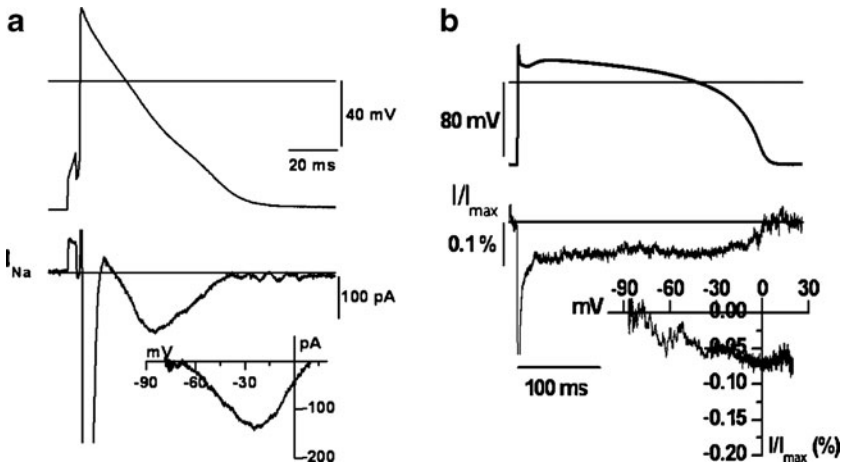

Fig. 2 TTX-sensitive current (*lower panels*, largely representative of I_{Na}) elicited by clamping the membrane with the action potential waveform (*upper panel*) (AP-clamp); the inset in each panel shows the dynamic I/V relation, obtained by plotting current as a function of membrane potential. TTX-sensitive current flowing during repolarization includes all sustained Na^+ components. **a** recording in a rat myocyte, clamped with its own action potential (Rocchetti et al. unpublished); **b** recording in a cell line (HEK239) transfected with NaV1.5 (α and β subunits) and clamped with a human ventricular action potential (from ref [12], modified). I_{NaT} is truncated in all recordings

conferring to this current "reverse" rate-dependency [17], which is partial at physiological rates (approx 50 % reduction from quiescence to 120 b/min) [18].

Mutations prolonging action potential duration (APD) cause enhancement of plateau Na^+ current through one or more of the mechanisms described above. For instance, all the above (widened voltage window, inactivation instability and accelerated recovery) may be operative in ΔKPQ mutants [1], but only non-equilibrium reactivation appears to account for the APD prolonging effect observed with the Il768V mutant [7].

Conditions and Mechanisms of I_{NaL} Enhancement

Although the interest in I_{NaL} pathophysiological role was mostly triggered by identification of Na^+ channel mutations leading to arrhythmogenic QT prolongation [1], secondary I_{NaL} enhancement occurs in association to a surprisingly large number of common disease states (reviewed in [19]), including cardiac hypertrophy/failure and ischemia, not necessarily related to each other in terms of primary pathogenesis. Such a pattern suggests that I_{NaL} enhancement may be a common response to cell stress/dysfunction. Accordingly, in cardiac myocytes, I_{NaL} is enhanced by reactive oxygen species (ROS) [20], whose generation is generally associated to cell distress. The question of whether ischemia can enhance I_{NaL} can be answered only indirectly, because membrane currents can be recorded only in isolated myocytes, which cannot be subjected to ischemia. Nevertheless, I_{NaL} is enhanced by myocyte exposure to hypoxia [21] or ischemic metabolites [22] and I_{NaL} blockade effectively prevents ischemia/reperfusion damage in the intact heart [23].

The search of a common mechanism, mediating I_{NaL} enhancement by cellular stress of heterogeneous etiology, has highlighted the role of Ca^{2+}-calmodulin kinase (CaMKIIδ) activation [24]. This is a Ca^{2+}- and ROS-activated cytosolic enzyme which may, among other targets, phosphorylates NaV1.5 channels. Converging evidence indicates that CaMKIIδ overexpression (or activation) enhances I_{NaL} and generates cardiac abnormalities that are reversed by I_{NaL} blockade [25]. Upstream components of the CaMKII activation pathway, i.e. calmodulin (CaM) and Ca^{2+} itself, have also been shown to enhance I_{NaL} directly, with differences between normal and failing myocytes [26]. While this does not rule out further modes of I_{NaL} enhancement (see ref [27]. for review), CaMKIIδ activation is particularly relevant because its relation with I_{NaL} may set up a vicious feed-back loop (Fig. 3), very likely to contribute to evolution of cell dysfunction and damage in response to stress. Whereas CaMKII inhibition is not available for therapeutic use yet, I_{NaL} blockade may break such a vicious loop [25].

To summarize, except for the case of primary (genetic) Na^+ channel defects, I_{NaL} enhancement might be viewed as a generic response to cellular stress, that is secondary in origin, but has a pivotal role in mediating functional derangements and disease progression.

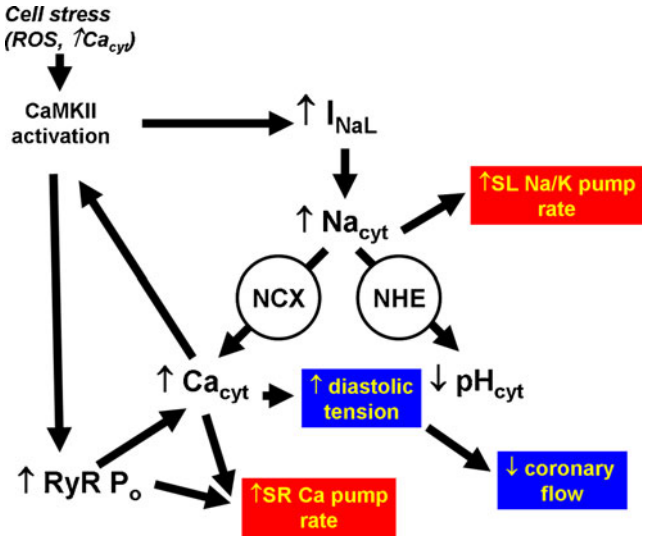

Fig. 3 Network of events linking I_{NaL} enhancements to its pleiotropic effects. Notably, many events are linked in positive feed back loops, likely to amplify and sustain the network once it has been initiated. Experimental observations (see text) suggest that I_{NaL} enhancement and CaMKII activation are key elements in this process. Events in *red boxes* imply increased ATP consumption, those in *blue boxes* reduced oxygen supply

I_{NaL} enhancement can also result from abnormalities in proteins other than the Na^+ channel itself, but interacting with it to form macromolecular complexes [28, 29]. For instance, mutations of scaffold and adaptor proteins, as ankyrin-B [30] and caveolin-3 [31] respectively, have been recently identified in LQT3 patients. A detailed review of the factors potentially contributing to I_{NaL} enhancement in heart failure has been provided by Matsev et al. [27].

I_{NaL} Impact on Electrical Activity and Ionic Homeostasis

I_{NaL} directly affects electrical activity (it promotes depolarization or counters repolarization) and provides a route of Na^+ influx. Considering the role of Na^+ gradient in transmembrane transport, the latter may affect cellular solute homeostasis, thus generating a host of indirect consequences of physiological and pathophysiological relevance.

Electrophysiological Effects

I_{NaL} flows during repolarization and may directly affect its course. In normal canine ventricles I_{NaL} is differentially expressed across the wall (M-cells and Purkinje cells > subendocardial cells > subepicardial cells) [17, 32, 33]. Thus, it is conceivably a player in the physiological transmural repolarization gradient and in its rate-dependency in

the dog (APD restitution) [34]. In normal ventricular myocytes, I_{NaL} inhibition by ranolazine (I_{Na} blocker with selectivity for I_{NaL} vs I_{NaT}) causes negligible APD changes. On the other hand, the remarkable effects of I_{Kr} blockade on APD, on its rate-depencency and, most importantly, on repolarization stability are all reversed by ranolazine [18, 35, 36]. These apparently contrasting findings may be reconciled by considering that ranolazine also partially blocks I_{Kr} [37]. Under basal conditions, this may offset the effect of I_{NaL} inhibition on APD; in turn, concomitant I_{NaL} inhibition limits the effect of I_{Kr} inhibition, thus preventing repolarization instability. If this interpretation is correct, we can conclude that I_{NaL} and I_{Kr} are physiologically in balance during normal repolarization; whenever this balance is altered, by either I_{NaL} enhancement or I_{Kr} blockade, repolarization stability is compromised. An extreme example of this condition is advanced heart failure, in which I_{NaL} enhancement and I_{Kr} downregulation coexist and are associated with dramatic repolarization instability [15]. Although the concept of I_{Kr} - I_{NaL} balance is valid in a broad sense, the effects of I_{Kr} blockade and I_{NaL} enhancement on action potential contour are not identical, an observation which may have its counterpart in the differences of clinical presentation between the LQT2 (I_{Kr} deficiency) and LQT3 (I_{NaL} enhancement) syndromes [38].

The direct contribution of I_{NaL} to repolarization course provides a first powerful mechanism linking arrhythmogenesis to I_{NaL} enhancement. Nevertheless, the latter may also facilitate arrhythmias through Ca^{2+} handling abnormalities (see below) and it is difficult to establish which mechanism prevails in a specific condition. The mutual interplay between Ca^{2+} handling and repolarization course [39] may actually make this distinction pointless.

A role of I_{NaL} in arrhythmogenesis is often inferred from the antiarrhythmic effect of its selective blocker ranolazine. While this may be considered legitimate in many cases, there are exceptions due to specificities of drug action. The best example is ranolazine efficacy on atrial arrhythmias, to which mechanisms other than I_{NaL} inhibition may also contribute [40].

Effects on Ionic Homeostasis

I_{Na} represents the main source of Na^+ entry during the cardiac cycle. Abeit I_{NaL} amplitude is normally 1/1000 of that of I_{NaT} [10], I_{NaL} persists throughout repolarization; as a result, I_{NaT} and I_{NaL} may similarly contribute to Na^+ influx during a cardiac cycle [41]. Nevertheless, I_{NaL} inhibition (by TTX or ranolazine) marginally affects Ca^{2+} cycling [42] and contractility [43] in normal hearts, thus suggesting that the attending changes in Na^+ influx are effectively buffered by matching changes in Na^+ extrusion. However, cellular homeostasis can be compromised by the marked increase in Na^+ influx resulting from pathological I_{NaL} enhancement.

Na^+ is normally extruded from the cell by the ATP powered Na^+/K^+ pump. Therefore excess Na^+ influx, even when successfully buffered, may increase ATP consumption. Furthermore, if influx exceeds the maximal extrusion rate, Na^+ accumulates in the cytosol, thus partially dissipating its transmembrane gradient. Because the latter energizes many secondary membrane transport mechanisms, most importantly the Na^+/Ca^{2+} exchanger (NCX) and the Na^+/H^+ exchanger (NHE), a pivotal consequence of I_{NaL} enhancement is perturbed homeostasis of intracellular Ca^{2+} and H^+ (Fig. 3).

NCX is the main mechanism of Ca^{2+} extrusion from the cell. Increased cytosolic Na^+ moves its electrochemical equilibrium potential in the negative direction, thus reducing the driving force for its forward operation during diastole and possibly reversing the direction of transport during systole (i.e. Ca^{2+} entry through NCX). The resulting increase in intracellular Ca^{2+} may re-establish NCX driving force, but the system equilibrium is now moved to higher cytosolic Ca^{2+} levels. Under conditions of I_{NaL} enhancement (e.g heart failure) NCX expression may be upregulated [44] and, provided that a driving force still exists, this may increase Ca^{2+} transport rate. However, the effect of this change in sustaining Ca^{2+} extrusion can only be partial, because it vanishes as NCX electrochemical equilibrium is approached. Accordingly, unless maximal Na^+/K^+ pump transport rate is also incremented, an increase in total cellular Ca^{2+} content is a necessary consequence of I_{NaL} enhancement. A further aspect of interest is the distribution of such an increment between subcellular compartments. Stimulation of Ca^{2+} uptake by the sarcoplasmic reticulum (SR) (by SERCA2) is a central component of physiological stimuli meant to increase cell Ca^{2+} content (e.g. by β-adrenergic activation). This ensures that most of the gain concerns SR luminal Ca^{2+}, which results in larger amplitude of Ca^{2+} transient (larger developed force) and lower diastolic Ca^{2+} (accelerated relaxation). This is not the case for I_{NaL} enhancement, in which the Ca^{2+} increment concerns primarily the cytosol, which may only secondarily increase Ca^{2+} in the SR. Although the latter may increase maximal developed force, this is expectedly associated with higher diastolic Ca^{2+}. The consequences of cytosolic Ca^{2+} being persistently elevated are multiple and of pathophysiological relevance.

Opening of SR Ca^{2+} release channels (RyRs) is directly triggered by cytosolic Ca^{2+}, a process facilitated by high Ca^{2+} in the SR lumen [45]. Increased cytosolic Ca^{2+} also activates CaMKII-dependent RyRs phosphorylation, a further mechanism of RyRs facilitation (see below, Fig. 3). It is therefore unsurprising that I_{NaL} enhancement may lead to facilitation of diastolic "Ca^{2+} waves" and the resulting electrical disturbances (delayed afterdepolarizations, DADs) [46]. This represents an important arrhythmogenic mechanism, probably the one prevailing under conditions of altered Ca^{2+} handling (e.g. heart failure).

With persistently elevated cytosolic Ca^{2+}, diastolic function may be hampered by delayed and incomplete sarcomere relaxation. A contribution of I_{NaL} to diastolic dysfunction has been demonstrated in various experimental models of heart failure [25, 42] and ranolazine improved diastolic relaxation in human ischemic heart disease [47]. In addition to its direct hemodynamic impact, diastolic dysfunction may limit coronary flow by extrinsic compression of intramural vessels. Significance of this effect is indirectly demonstrated by the ability of ranolazine to improve myocardial perfusion in the setting of coronary artery disease (likely I_{NaL} enhancement) [48]. Indeed, being ranolazine devoid of significant direct vasodilator effects, the perfusion improvement is likely to result from accelerated diastolic relaxation, a well known factor in modulation of coronary flow [49].

Abnormally elevated cytosolic Ca^{2+} may also activate a number of signalling pathways involved in modulation of cell function and, in the long run, structure and fate. CaMKIIδ activation, is at the same time, a cause (see above) and a consequence [50] of I_{NaL} enhancement. Phosphorylation of SR Ca^{2+} release channels (RyRs) by this kinase increases their open probability [51], ultimately leading to destabilization of the Ca^{2+} store. This accounts for the facilitation of spontaneous Ca^{2+} release events and delayed afterpotentials (DADs, their arrhythmogenic electrical consequence) induced by either I_{NaL} enhancement or CaMKIIδ overexpression [52] and suppressed in both cases by I_{NaL} blockade [25]. Calcineurin, a cytosolic Ca^{2+}-activated phosphatase, regulates translocation to the nucleus of NFAT, a transcriptional regulator centrally involved in hypertrophic remodelling [53]. While there is still no direct evidence for the involvement of this specific pathway in I_{NaL}-induced damage, ranolazine has been shown to affect downstream gene transcription and histomorphometric parameters in heart failure induced by coronary microembolization [54].

A further consequence of pathological I_{NaL} enhancement is a derangement in cell energy balance. The main ATP-consuming mechanism involved in the control of cellular homeostasis (the Na^+/K^+ and SERCA pumps) are conceivably short-circuited by enhanced sarcolemmal Na^+ influx and increased Ca^{2+} leak from the SR (by RyRs facilitation) (Fig. 3). Therefore, I_{NaL} enhancement is expected to increase ATP consumption by mechanisms not directly involved in force generation, which would translate into decreased mechanical efficiency. This view is supported by the observation that I_{NaL} blockade prevents the drop in myocardial ATP, but not the positive inotropic effect, of ouabain [55]. In addition to improved coronary perfusion, increased myocardial efficiency might partly account for the ability of I_{NaL} blockade to improve exercise capacity of ischemic patients without altering systolic mechanical work [56].

It has been reported that increased cytosolic Na^+ may also jeopardize mitochondrial function (Fig. 4). Concomitant cytosolic Na^+ loading (to 15 mM) impaired Ca^{2+}-triggered

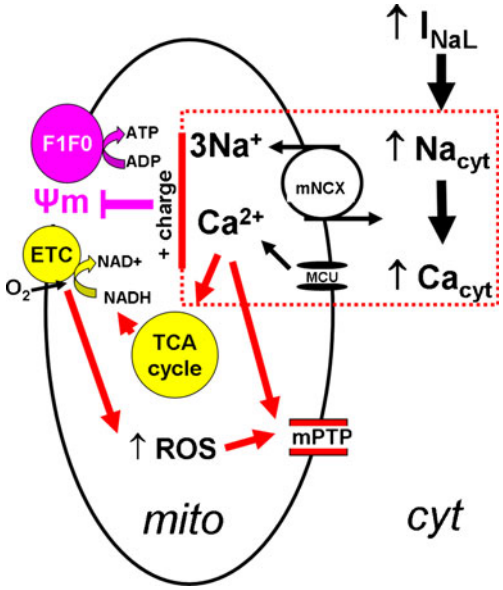

Fig. 4 A plausible sequence of events coupling I_{NaL} enhancement to mitochondrial damage. Functions primarily depressed upon I_{NaL} enhancement are in *purple*, those upregulated to compensate in *yellow*. Elevated cytosolic Ca^{2+} (Ca_{cyt}) loads the mitochondria through the Ca^{2+} uniporter (MCU) and stimulates NADH production by the tricarboxilic acids (TCA) cycle. Excess mitochondrial Ca^{2+} is partially removed by mitochondrial NCX (mNCX), driven by high cytosolic Na^+ [57]. This chain of events (in the *dotted box*) results in positive charge influx, which tends to dissipate the electrical gradient (Ψm) that drives ATP synthesis by the ATP-synthase (F1F0) [64]. The resulting drop in the ATP/ADP ratio is compensated by increasing the electron transport rate (by ETC), fuelled by NADH [57]. In this scheme NADH and ATP levels may be preserved up to a limit [57], but at the cost of increased substrate consumption and ROS production by ETC [43]. Concomitantly elevated mitochondrial Ca^{2+} and ROS facilitate opening of mPTP [43], eventually resulting in loss of membrane selectivity, swelling and disruption of the outer mitochondrial membrane. Release of cyrochrome C and other components of the outer membrane space triggers cell apoptosis [64]

mitochondrial energetic adaptation in patch-clamped myocytes, thereby causing an abnormal drop in NADH (i.e metabolic energy) upon catecholamine challenge [57]. On the other hand, ranolazine pre-treatment failed to modify NADH course during global ischemia/reperfusion in isolated hearts [43]. Notably, in the same preparation, ranolazine opposed ischemia-induced rise in cytosolic and mitochondrial Ca^{2+}; this was associated, as expected, with diminished ROS generation and delayed opening of mitochondrial permeability transition pore (mPTP) [43]. A plausible network of mechanisms in accord with these observations is illustrated in Fig. 4. Altogether, this preliminary evidence suggests that, during ischemia/reperfusion, I_{NaL} inhibition may be more effective in preserving integrity of mitochondria than in improving their function. Although ranolazine effects on energy metabolism can be accounted for by I_{NaL} inhibition, the drug has been also reported to inhibit fatty acid oxidation [58, 59], a further action potentially protecting mitochondria during metabolic stress.

However, this action was observed with drug dosage (60–200 mg/Kg/day) far from that required for the anti-ischemic effect in humans (10–15 mg/Kg/day).

Under normal conditions, cellular H^+ homeostasis may be less sensitive than Ca^{2+} homeostasis to I_{NaL} enhancement, because mechanisms other than NHE contribute to H^+ extrusion. During abrupt reperfusion following acute coronary occlusion, massive H^+ extrusion through NHE contributes to intracellular Na^+ loading, which explains why direct NHE inhibition paradoxically improves Ca^{2+} handling and limits myocardial injury [60]. Nevertheless, the situation might be different in the presence of chronic partial ischemia, when reduced H^+ export by NHE might lead to persistent intracellular acidosis, potentially contributing to impair force development and relaxation. Although awaiting experimental confirmation, this hypothesis is supported by the observation that the increment in intracellular Na^+ caused by Na^+/K^+ pump blockade does produce intracellular acidosis, which can be prevented by concomitant I_{NaL} blockade [55].

Functional Interaction Between I_{NaL} and K^+ Currents

Several observations suggest a complex interplay, with partly unexplained mechanisms, between I_{NaL} and K^+ currents during repolarization. Such an interplay may have consequences on electrophysiology and ionic homeostasis.

The impact on repolarization of blocking endogenous I_{NaL} in the presence of K^+ current inhibition (I_{Kr}, I_{to} or I_{K1}) [36] may look disproportionately large to the small magnitude of the inward current removed by I_{NaL} block. Although ranolazine, often used as the I_{NaL} blocker, shares the channel binding domain on HERG channels (carrying I_{Kr}) with E-4031, it cannot competitively displace E-4031 [36]. Moreover, ranolazine effects are largely mimicked by TTX, which does not bind to HERG channels. Therefore, this disproportion cannot be attributed to ancillary ranolazine properties and remains largely unexplained.

A further relevant aspect of I_{NaL} blockade is its ability to interfere with the intrinsic reverse rate-dependency of APD and of its amplification by K^+ channel blockers [18]. Because I_{NaL} decreases at faster rates (see above), I_{NaL} block shortens APD more at slower ones, thus dampening the intrinsic reverse rate-dependency of repolarization [18]. Rate-dependency of I_{NaL} may also account for the ability of I_{NaL} block to flatten "APD restitution" curves, an action strongly correlated with antifibrillatory effect [34].

Recent work on olfactory neurons highlights a structurally organized relationship between Na^+ channels and the Na^+-activated K^+ current (I_{KNa}) [61], whereby the latter is specifically activated by Na^+ influx through I_{NaL}. I_{KNa} activation may oppose I_{NaL}-induced membrane depolarization but, by doing this, it would increases the driving force for Na^+ influx. Thus, coupling to I_{KNa} might partially dissociate the effects of I_{NaL} enhancement on membrane potential from those on intracellular ionic homeostasis. I_{KNa} is expressed in (guinea-pig) ventricular myocytes [62] and contributes to regulate APD [63]. However, its structural association with cardiac I_{Na} channels and functional interaction with I_{NaL} enhancement has not been investigated thus far.

Conclusions

Although of small magnitude, endogenous I_{NaL} contributes in setting repolarization course of normal myocytes, being in a delicate balance with K^+ currents. Disruption of this balance may strongly affect repolarization and its stability. Furthermore, I_{NaL} enhancement, occurring in many pathological conditions as a general response to cell stress, can profoundly alter intracellular ionic homeostasis with consequences on contractile function, electrical stability and cell fate. Accordingly, I_{NaL} represents a therapeutic target with an expectedly pleiotropic effect, which is being gradually unveiled by experimental and clinical studies.

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